

In the Specification

Please replace the paragraph at page 6, lines 14 through 15 with the following paragraph:

B¹ Figure 19 shows FACS analysis of propidium iodide stained cells, treated with 40 μ M of the indicated oligonucleotide.

Please replace the paragraph at page 10, lines 14 through 22 with the following paragraph:

B² Example 10 also demonstrates that pTpT induces production of IL-10 mRNA and protein which is active in inhibiting T cell proliferation in allogenic mixed lymphocyte assay. In human skin, IL-10 as well as TNF α induce specific tolerance for contact hypersensitivity and delayed-type hypersensitivity reactions. Therefore, the DNA fragments, oligonucleotides, deoxynucleotides, dinucleotides and dinucleotide dimers of the present invention are reasonably expected to have immunosuppressive effects *in vivo*, e.g., to inhibit contact hypersensitivity and delayed-type hypersensitivity. These findings expand the spectrum of UVB effects mimicked by the compounds of the present invention.

Please replace the paragraph at page 25, line 7 to page 26, line 3 with the following paragraph:

B³ Both the GADD 45 and SDI 1 genes are known to be transcriptionally regulated by the tumor suppressor protein p53. After UV- and γ -irradiation, as well as treatment of cells with DNA-damaging chemical agents, there is a rapid stabilization and nuclear accumulation of p53 after which this protein binds to specific promoter consensus sequences and modulates the transcription of regulated genes. Recent data suggest that p53 can also be activated by the binding of small single-stranded DNAs, as well as certain peptides and antibodies, to a carboxyl terminal domain of this protein. In order to determine whether the inhibitory effect of the

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dinucleotide pTpT on cell proliferation is mediated through p53, the growth response of a p53 null cell line, H1299 lung carcinoma cells, was examined. The p53-null H1299 cells (Sanchez, Y. *et al.*, *Science* 271:357-360 (1996)) were maintained in DMEM with 10% calf serum. Preconfluent cultures were given fresh medium supplemented with either 100 μ M pTpT or diluent. Cells were collected on consecutive days by trypsinization, and counted by CoulterTM counter. As shown in Figure 9, there was no inhibition of proliferation of pTpT-treated H1299 cells compared to diluent-treated controls.

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Please replace the paragraph at page 27, line 18 through page 28, line 13 with the following paragraph:

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Newborn keratinocytes were established as described (Stanulis-Praeger, B.M. and Gilchrest, B.A., *J. Cell. Physiol.* 139:116-124 (1989)) using a modification of the method of Rheinwald and Green (Gilchrest, B.A. *et al.*, *J. Invest. Dermatol.* 101:666-672 (1993)). First-passage keratinocytes were maintained in a non-differentiating low Ca²⁺ medium (K-Stim, Collaborative Biomedical Products, Bedford, MA). Fibroblasts were established from dermal explants as described (Rheinwald, J.G. and Green, J., *Cell* 6:331-343 (1975)) and maintained in DMEM supplemented with 10% bovine serum. Cells were treated with either 100 μ M pTpT or an equal volume of diluent (DMEM) for five days prior to transfection. Duplicate cultures of each condition were transfected using the Lipofectin Reagent Kit (GIBCO/BRL) and 5 μ g reporter DNA, pCAT-control vector (Promega, Madison, WI). Before transfection, the vector DNA was either sham irradiated or exposed to 100 mJ/cm² UVB radiation from a 1 KW Xenon arc solar simulator (XMN 1000-21, Optical Radiation, Azuza, CA) metered at 285 \pm 5 nm using a research radiometer (model IL 1700A, International Light, Newburyport, MA), as described (Yaar, M. *et al.*, *J. Invest. Dermatol.* 85:70-74 (1985)). Cells were collected 24 hours after transfection in a lysis buffer provided in the CAT Enzyme Assay System (Promega, Madison, WI) using a protocol provided by the manufacturer. CAT enzyme activity was determined using the liquid scintillation counting protocol and components of the assay system kit. Labeled chloramphenicol [50-60 mCi (1.85-2.22 GBq/mmol)] was purchased from New England Nuclear

B⁴ (Boston, MA). Protein concentration in the cell extracts was determined by the method of Bradford (Anal. Biochem. 72:248 (1986)). CAT activity was expressed as c.p.m./100 µg protein and is represented as percent activity of cells transfected with sham-irradiated, non-damaged, plasmid.

Please replace the paragraph at page 35, line 20 through page 36, line 4 with the following paragraph:

B⁵ pTpT, shown previously to stimulate pigmentation in these cells, was used as a reference treatment and diluent alone as a negative control. After five days of treatment with the oligonucleotides, the cells were collected, counted, and an equal number of cells were pelleted for melanin analysis. The data shown in Figure 17 demonstrate that 10 µM pTpT increased melanin content to 3 times that of control diluent-treated cells. SEQ ID NO: 5, representing the telomere over-hang sequence, also at 10 µM, increased the melanin level to 10 times that of control cells. SEQ ID NO: 9 (telomere over-hang complement) and SEQ ID NO: 10 (unrelated sequence) did not produce significant change in pigment content at concentration up to 10 µM. A truncated version of SEQ ID NO: 5, comprising TTAGGG (SEQ ID NO: 11) was also highly melanogenic, while the reverse complimentary sequence CCCTAA (SEQ ID NO:12) was less active (Figure 18), where both oligonucleotides contained a 5' phosphate).

Please replace the paragraph at page 36, lines 11 through 17 with the following paragraph:

B⁶ In another experiment, mice were treated once daily with either 100 µM pTpT or SEQ ID NO: 1 containing a 5' phosphate in propylene glycol on one ear, or vehicle alone on the other ear. After 15 days, when the ears were sectioned and stained with Fontana Masson to detect melanin compared to vehicle controls, there was a 70% increase in pigmentation in pTpT-treated ears and

β^6 a 250% increase with SEQ ID NO: 1. Thus, both compounds comprising as few as 2 and as many as 9 nucleotides are effective at producing the *in vitro* UV-mimetic effects *in vivo*.

Please replace the paragraph at page 38, line 2 through page 39, line 3 with the following paragraph:

β^7 Fluorescein phosphoramidite (FAM) labeled oligonucleotides were added to cultures of S91 cells for 4 hours and the cells were then prepared for confocal microscopy. Nuclei, identified by staining with propidium iodide, appeared red and FAM-labeled oligonucleotides appeared green. Co-localization of red and green signals was assigned a yellow color by the computer. Oligonucleotides with a 5' phosphate showed greater cellular uptake than those lacking this moiety. Confocal microscopy failed to detect uptake of TpT and fluorescence-activated cell sorting (FACS) analysis of these cells and gave a profile similar to that seen with untreated cells. pTpT-treated cells showed strong green fluorescence in the cytoplasm, but only a small amount of nuclear localization. FACS analysis showed a shift in the peak fluorescence intensity, compared to TpT-treated cells, indicating more intensely stained cells. Similarly, the presence of the phosphate at the 5' end of SEQ ID NO: 1 greatly enhanced its uptake into the S91 cells. SEQ ID NO: 1 without 5' phosphorylation showed only moderate uptake and was localized predominantly in the cytoplasm, with faint nuclear staining in only some cells, whereas SEQ ID NO: 1 with 5' phosphorylation showed intense staining that strongly localized to the nucleus. FACS analysis of SEQ ID NO: 1 without 5' phosphorylation showed a broad range of staining intensities with essentially two populations of cells, consistent with the confocal images. The phosphorylated SEQ ID NO: 1 containing cells also showed a range of staining intensities, but with more cells showing higher fluorescent intensity. Cells treated with phosphorylated SEQ ID NO: 8 showed a pattern of fluorescence very similar to that seen with phosphorylated SEQ ID NO: 1, both by confocal microscopy and FACS analysis, indicating that its lower activity in the melanogenesis assay cannot be ascribed to poor uptake. These data show that uptake of these oligonucleotides by S91 cells is greatly facilitated by the presence of 5' phosphate and that melanogenic activity, while consistent with a nuclear site of action, is not solely dependent on

B⁷ nuclear localization. Also, although the total intracellular fluorescence did not increase appreciably with increasing oligonucleotide length among the DNAs tested, the larger oligonucleotides more readily accumulated in the cell nucleus. There was no change in the profile of oligonucleotide uptake after 6 and 24 hours.

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Please replace the paragraph at page 39, lines 5 through 23 with the following paragraph:

B⁸ Oligonucleotides homologous to the telomere overhang repeat sequence (TAAGGG) sequence (11mer-1: SEQ ID NO: 5), complementary to this sequence (11mer-2: SEQ ID NO: 9) and unrelated to the telomere sequence (11mer-3: SEQ ID NO: 10) were tested. The three 11-mer oligonucleotides were added to cultures of Jurkat cells, a line of human T cells, one of the cell types reported to undergo apoptosis in response to telomere disruption. Within 48 hours, 50% of the cells treated with 40 μ M of SEQ ID NO:5 had accumulated in the S phase, compared to 25-30% for control cells ($p < 0.0003$, non-paired t-test), and by 72 hours, 13% of these cells were apoptotic as determined by a sub-G₀/G₁ DNA content, compared to 2-3% of controls ($p < 0.007$, non-paired t-test) (Fig. 19). At 96 hours, 20+3% of the 11mer-1 treated cells were apoptotic compared with 3-5% of controls ($p < 0.0001$, non-paired t-test). To exclude preferential uptake of the 11mer-1 as an explanation of its singular effects, Jurkat cells were treated with oligonucleotides labelled on the 3' end with fluorescein phosphoramidite, then subjected to confocal microscopy and FACS analysis. The fluorescence intensity of the cells was the same after all treatments at 4 hours and 24 hours. Western analysis showed an increase in p53 by 24 hours after addition of 11mer-1, but not 11mer-2 or -3, with a concomitant increase in the level of the E2F1 transcription factor, known to cooperate with p53 in induction of apoptosis and to induce a senescent phenotype in human fibroblasts in a p53-dependent manner as well as to regulate an S phase checkpoint.

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i - vi).